इंटरनेट मानक

Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

“जानने का अधिकार, जीने का अधिकार”
Mazdoor Kisan Shakti Sangathan
“The Right to Information, The Right to Live”

“पुराने को छोड नये के तरफ”
Jawaharlal Nehru
“Step Out From the Old to the New”

IS 15697-2 (2006): Chillies and chilli oleoresins - Determination of total capsaicinoid contents, Part 2: Method using high-performance liquid chromatography [FAD 9: Spices and Condiments]

“ज्ञान से एक नये भारत का निर्माण”
Satyanarayan Gangaram Pitroda
“Invent a New India Using Knowledge”

Act Number 22 of 2005
The Indian Parliament
RIGHT TO INFORMATION

Made Available By
Public.Resource.Org

“ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है”
Bhartṛhari—Nītiśatakam
“Knowledge is such a treasure which cannot be stolen”

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

IS 15697 (Part 2) : 2006
ISO 7543-2 : 1993

भारतीय मानक

मिर्च और चिली ओलीओरेजिन — कैपसीसिनोईड का कुल अंश ज्ञात करना

भाग 2 उच्च-कार्यकारिता द्रव क्रोमेटोग्राफी प्रयुक्त पद्धति

Indian Standard

CHILLIES AND CHILLI OLEORESINS — DETERMINATION OF TOTAL CAPSAICINOID CONTENT

PART 2 METHOD USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ICS 67.220.10

© BIS 2006

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

November 2006

Price Group 3

Spices and Condiments Sectional Committee, FAD 9

NATIONAL FOREWORD

This Indian Standard (Part 2) which is identical with ISO 7543-2 : 1993 'Chillies and chilli oleoresins — Determination of total capsaicinoid content — Part 2 : Method using high-performance liquid chromatography' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Spices and Condiments Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 2825 : 1981 Spices and condiments — Preparation of a ground sample for analysis	IS 1797 : 1985 Method of test for spices and condiments	Technically Equivalent

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CHILLIES AND CHILLI OLEORESINS — DETERMINATION OF TOTAL CAPSAICINOID CONTENT

PART 2 METHOD USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

1 Scope

This part of ISO 7543 specifies a method for the determination, by high-performance liquid chromatography, of the total capsaicinoid content of whole or powdered chillies (usually *Capsicum frutescens* L.) and their extracts (oleoresins). This content is calculated from the total of capsaicin, nordihydrocapsaicin and dihydrocapsaicin, expressed as nonyl acid vanilylamide, which is the chosen reference substance.

This method enables the separation of capsaicin and nonyl acid vanilylamide.

NOTE 1 A spectrometric method is given in ISO 7543-1.[1]

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 7543. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 7543 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 2825:1981, *Spices and condiments — Preparation of a ground sample for analysis.*

3 Principle

3.1 Chillies in powder form

Extraction with tetrahydrofuran using a Soxhlet-type continuous extraction apparatus, then determination of the capsaicinoids by high-performance liquid chromatography (HPLC), in accordance with the procedure described in this part of ISO 7543.

3.2 Whole chillies

Preparation by grinding the sample, then extraction of the powder so obtained, followed by determination of the capsaicinoids by HPLC, in accordance with the procedure described in this part of ISO 7543.

3.3 Oleoresins of chillies

Dilution of the oleoresin in a tetrahydrofuran/methanol solution, then determination of the capsaicinoids by HPLC, in accordance with the procedure described in this part of ISO 7543.

4 Reagents

Use only reagents of recognized analytical grade, and distilled or demineralized water or water of equivalent purity.

---

[1] To be published.

4.1 Reference substance

Nonyl acid vanilylamide (synthetic capsaicin) of at least 95 % purity, determined by chromatography under the test conditions given in this part of ISO 7543.

WARNING — This product should be handled carefully as it is strongly irritating.

4.2 Solvents

4.2.1 Tetrahydrofuran

4.2.2 Methanol

4.2.3 Silver nitrate, aqueous solution, 0,1 mol/l.

WARNING — This product should be handled with care as skin contact will cause black spots.

4.2.4 Tetrahydrofuran/methanol solution, 50/50 (*V*/*V*).

4.3 Elution solvent

Mix 70 volumes of methanol (4.2.2) and 30 volumes of silver nitrate (4.2.3).

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Pipettes, of 5 ml capacity.

5.2 Volumetric flasks, of 25 ml and 100 ml capacity.

5.3 Filtration diaphragm, made of cellulose acetate, of diameter 25 mm and pore size 0,45 µm.

5.4 Separation system

5.4.1 Chromatograph, for high-performance liquid chromatography.

5.4.2 Pumping system, which enables a flow rate which is constant or programmed at high pressure to be obtained and maintained.

5.4.3 Degassing systems, for solvents.

5.4.4 Suitable detector system, adjustable to a wavelength of 280 nm.

5.5 Recorder or **integrator**, the performance of which is compatible with the apparatus as a whole.

5.6 Column

Material: stainless steel

Length: 10 cm to 25 cm

Inside diameter: 0,4 cm to 0,5 cm

Stationary phase: grafted silica with derived octadecyl functional group, C_{18}, and particle size 5 µm max.

5.7 Injection system

Injection valve with 10 µl loop or any other system giving the same injection accuracy.

5.8 Continuous-extraction apparatus, Soxhlet type.

5.9 Test sieve, with aperture size 500 µm.

6 Calibration method

6.1 Reference solution

Prepare a stock solution of 1 g/l of nonyl acid vanilylamide (4.1) in the tetrahydrofuran/methanol solution (4.2.4).

6.2 Calibration curve

From the reference solution (6.1), prepare at least three standard solutions of nonyl acid vanilylamide with concentrations ranging from 0,05 g/l to 0,2 g/l. Inject each solution into the chromatograph (5.4.1). Repeat the determination at least once. Measure the peak areas and repeat the tests if the results deviate by more than 5 %.

Plot the calibration curve, i.e. the mass of the nonyl acid vanilylamide injected versus the peak area.

Define the mean slope of the curve.

6.3 Calculation of the response factor, *K*

Calculate the response factor K using the following formulae:

$$K = m'/A$$

$$m' = m \times P_r$$

where

m is the mass of nonyl acid vanilylamide, in milligrams;

A is the area of the peak of nonyl acid vanilylamide;

P_r is the purity of the nonyl acid vanilylamide (4.1);

m' is the corrected mass of nonyl acid vanilylamide, in milligrams.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 7543. A recommended sampling method is given in ISO 948[2].

8 Preparation of test sample

8.1 Chillies in powder form

8.1.1 Check that all of the test sample passes through the 500 µm sieve (5.9). If this is not the case, grind the powder in accordance with ISO 2825 until the required particle size is obtained.

8.1.2 From the test sample prepared as in 8.1.1, weigh, to the nearest 0,1 g, between 10 g and 15 g. Extract for 8 h in a continuous-extraction apparatus (5.8), using 100 ml of tetrahydrofuran (4.2.1).

Partially concentrate the solvent.

Quantitatively transfer the extract to a 100 ml one-mark volumetric flask (5.2). Make up to the mark with the tetrahydrofuran/methanol solution (4.2.4).

8.2 Whole chillies

8.2.1 Grind the test sample in accordance with ISO 2825 until the powder obtained passes entirely through the 500 µm sieve (5.9).

8.2.2 Using the test sample prepared as in 8.2.1, proceed as described in 8.1.2.

8.3 Oleoresins of chillies

Thoroughly homogenize the test sample of oleoresin.

9 Procedure

9.1 Test portion

9.1.1 Chillies, whole or in powder form

Take with the pipette (5.1) 5 ml of the extraction solution prepared as in 8.1.2 or 8.2.2 and transfer it to a 25 ml one-mark volumetric flask (5.2). Make up to the mark with the tetrahydrofuran/methanol solution (4.2.4).

NOTE 2 This dilution may be modified, if necessary, by the analyst depending upon the assumed total capsaicinoid content of the sample and the sensitivity of the detector.

9.1.2 Oleoresins of chillies

Weigh into a 100 ml one-mark volumetric flask, to the nearest 0,1 mg, 1 g of oleoresin (see 8.3) and make up to the mark with the tetrahydrofuran/methanol solution.

Pipette 5 ml of this solution into a 25 ml one-mark volumetric flask and make up to the mark with the elution solvent (4.3).

NOTE 3 This dilution may be modified, if necessary, by the analyst depending upon the assumed total capsaicinoid content of the sample and the sensitivity of the detector.

9.2 Determination

The test solution shall be clear. If this is not the case, filter it on the filter diaphragm (5.3). Inject the test solution into the chromatograph (5.4.1).

On the chromatogram obtained, measure the area of the peaks of capsaicin (C_1), nordihydrocapsaicin (C_2) and dihydrocapsaicin (C_3) (see typical chromatograms given in annex A).

Carry out two tests and repeat the determination if the results deviate by more than 5 %.

10 Calculation

Calculate the total capsaicinoid content, w_T, as a percentage by mass, using the following formula:

$$w_T = \frac{(A_{C1} + A_{C2} + A_{C3})K}{m_x} \times 500 \times 100$$

where

A_{C1} is the area of the capsaicin peak;

A_{C2} is the area of the nordihydrocapsaicin peak;

A_{C3} is the area of the dihydrocapsaicin peak;

m_x is the mass of the test portion, in milligrams;

K is the response factor determined for the reference substance.

NOTE 4 The dilution factor of 500 in this formula may be adjusted by the analyst according to the capsaicinoid content of the sample to be tested.

---

[2] ISO 948:1980, *Spices and condiments — Sampling.*

11 Repeatability

Take as the result for the response factor K and the content of the compound to be determined, the mean value of several (at least three) determinations carried out on the same test sample. These different values should not differ from their mean by more than 5 %.

12 Test report

The test report shall include the following information:

a) reference to this part of ISO 7543;

b) the apparatus system;

c) the characteristics of the column (material, dimensions, packing, stationary phase);

d) the characteristics of the detector (optional) and the operating conditions;

e) the characteristics of the mobile phase (flow rate and nature);

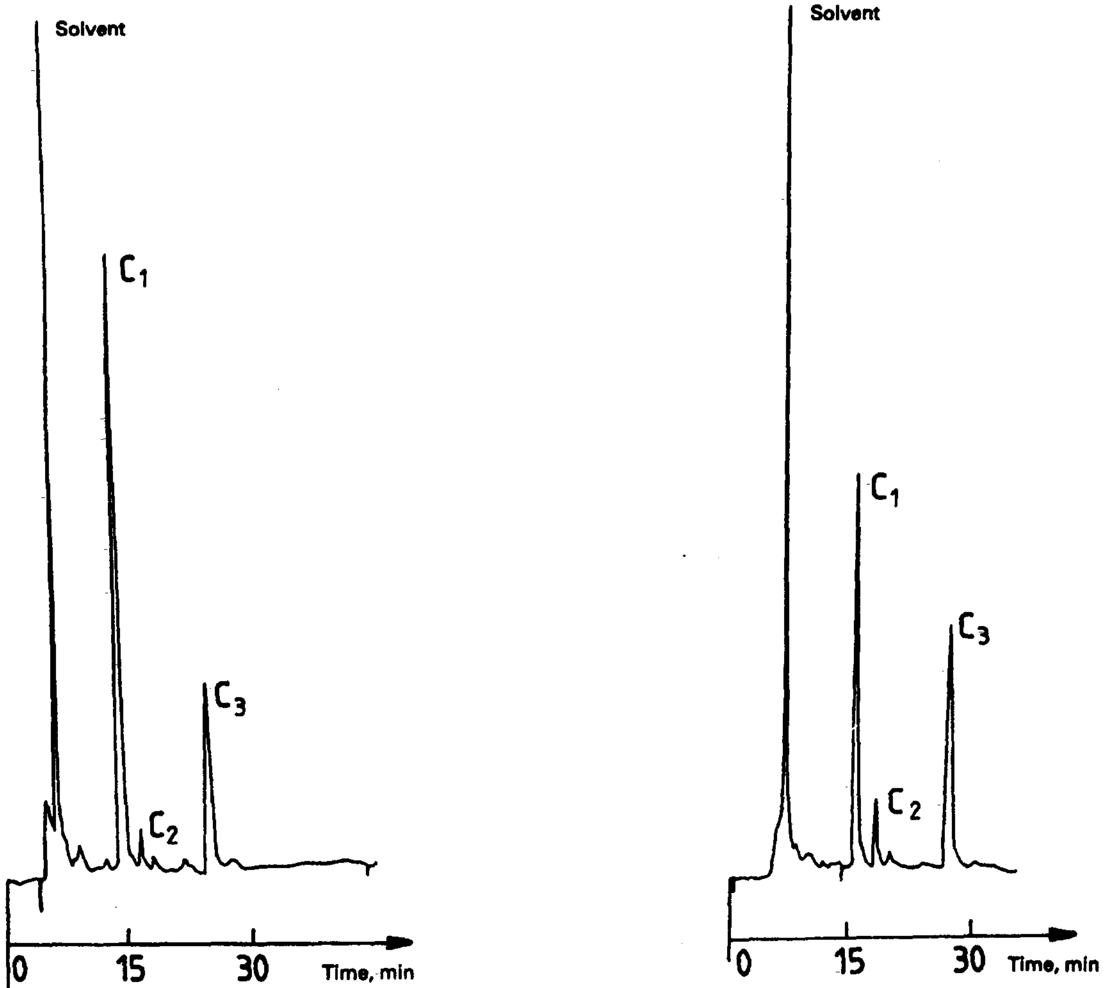
f) identification of the test sample (quantity injected and final dilution);

g) results obtained.

Annex A
(informative)

Typical chromatograms

Figure A.1 shows two examples of typical chromatograms taken under operating conditions given in this part of ISO 7543.



Oleoresin 1

Oleoresin 2

Identification of the peaks

C_1 Capsaicin
C_2 Nordihydrocapsaicin
C_3 Dihydrocapsaicin

Figure A.1

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in the course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

This Indian Standard has been developed from Doc: No. **FAD 9 (1423)**.

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones: 2323 0131, 2323 3375, 2323 9402 *website* : www.bis.org.in

Regional Offices:

		Telephones
Central	: Manak Bhavan, 9 Bahadur Shah Zafar Marg NEW DELHI 110002	2323 7617 2323 3841
Eastern	: 1/14 C.I.T. Scheme VII M, V.I.P. Road, Kankurgachi KOLKATA 700054	2337 8499, 2337 8561 2337 8626, 2337 9120
Northern	: SCO 335-336, Sector 34-A, CHANDIGARH 160022	260 3843 260 9285
Southern	: C.I.T. Campus, IV Cross Road, CHENNAI 600113	2254 1216, 2254 1442 2254 2519, 2254 2315
Western	: Manakalaya, E9 MIDC, Marol, Andheri (East) MUMBAI 400093	2832 9295, 2832 7858 2832 7891, 2832 7892

Branches: AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. NALAGARH. PATNA. PUNE. RAJKOT. THIRUVANANTHAPURAM. VISAKHAPATNAM.

Printed at Simco Printing Press, Delhi